

obtained in one fraction exhibited a mol.wt of 228 with an m/e at 119 for  $\text{H}_2\text{O}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{C}^+=\text{S}$ . NMR and IR spectral analysis and comparison with an authentic sample of thiarubrine-A established the structure of the oil as 1-(2-methyleth-1-yn)-4-(hex-1,3-diyn-4-ene)-2,3-dithiacyclohex-1,3 diene (**I**)<sup>8,9</sup>, previously isolated and identified from *Iva*<sup>7</sup>, *Ambrosia*<sup>7</sup>, *Schkuhria*<sup>7</sup>, *Palafoxia*<sup>7</sup>, *Eriophyllum*<sup>9</sup> and *Chaenactis*<sup>9</sup>. Although many species of the Asteraceae are used for a variety of medicinal purposes, the Okanagan-Colville Indians of British Columbia, Canada and Washington, USA, used *Chaenactis douglassii* var. *achilleaefolia* which contain thiarubrine A to treat wound infections and sores and as an eyewash<sup>10</sup>. Thiarubrine A has been found to be as effective as the strong photosensitizer,  $\alpha$ -terthienyl<sup>11</sup> against *Candida albicans*, *Staphylococcus albus*, *Mycobacterium phlei*, *Bacillus subtilis*, *Streptococcus faecalis* and *E.coli* either in UV-A or in light in the range of 0.1–1.0 ppm<sup>8</sup>. It is as effective against *Candida albicans* as fungizone at a concentration of 1 ppm in dark or 0.1

ppm in light. At 10 ppm it causes 100% mortality in the free living nematode *Coenorhabditis elegans*. It is also toxic to Chinese hamster ovary cells at 4 ppm in dark and phototoxic at 0.25 ppm<sup>11</sup>. It is therefore a potent biocidal agent. A rough estimate of the amount in a single leaf would be 5 mg, which would be the amount ingested by a chimpanzee per diem. The compound is unstable under acidic conditions, being converted to the corresponding thiophene<sup>9</sup>. Its metabolic fate in the GI tract of the chimpanzee is therefore of interest.

The medicinal use of species of *Aspilia* by Africans to cure sores and other skin infections would appear to make sense since thiarubrine A is such a strong antibiotic. *Aspilia mossambicensis* has been used in east Africa for the treatment of abdominal pains and intestinal worms, including hookworm<sup>12</sup>. Wild chimpanzees harbor many of the nematodes, trematodes and protozoans common to man<sup>13,14</sup>. Whether thiarubrine A is an effective oral antibiotic or anthelmintic awaits further investigation.

- 1 We thank Dr P. Kuchar (Somalia) for the collection of *A. pluriseta*. The work at the University of California was supported by the National Institute of Health (AI 18398 and AI 00472) and a UCI Research Travel Grant. The antibiotic studies at the University of British Columbia are financially supported by the Natural Sciences and Engineering Research Council of Canada.
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0014-4754/85/030419-02\$1.50 + 0.20/0  
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## Chemical attraction of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae)<sup>1</sup>

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**Summary.** Workers and queens of the eastern yellowjacket, *Vespula maculifrons*, are attracted to the artificial long-range attractant pheromone of the predaceous pentatomid, *Podisus maculiventris*. A 1:1 mixture of linalool or  $\alpha$ -terpineol and (*E*)-2-hexenal is as attractive to *V. maculifrons* workers as the pheromone.

**Key words.** Yellowjacket, eastern; *Vespula maculifrons*; attraction, chemical; pheromone, attractant; *Podisus maculiventris*.

Heptyl butyrate, 2,4-hexadienyl butyrate and a variety of similar esters are potent and specific attractants for *Vespula pensylvanica* and other yellowjackets in the western United States<sup>2,3</sup>, but these attractants are ineffective for eastern yellowjackets<sup>4,5</sup>. While field-testing a synthetic aggregation pheromone for the predaceous spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae), we noticed that pheromone-baited traps caught many more *Vespula* spp. than unbaited traps. Components of the *P. maculiventris* aggregation pheromone<sup>6</sup> were field-tested singly and in pairs to determine which compounds are attractive to yellowjackets.

**Methods and materials.** Components of the *P. maculiventris* aggregation pheromone (indicated by an asterisk in the table) and the other compounds tested were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Bedoukian Research Inc.

(Danbury, CN) except the enantiomers of  $\alpha$ -terpineol and (*E*)-2-hexenyl crotonate. (+)- $\alpha$ -Terpineol and (–)- $\alpha$ -terpineol were synthesized from (+)- $\alpha$ -pinene (Aldrich Chem.) and (–)- $\alpha$ -pinene (Tridom Chem., Hauppauge, NY)<sup>7</sup>. (*E*)-2-Hexenyl crotonate was synthesized from (*E*)-2-hexenol and crotonic acid by standard methods.

Field-tests for yellowjacket attraction were conducted in and around deciduous woods at the Agricultural Research Center-East, Beltsville, Maryland, during 1982 and 1983. Sticky wing traps (Zoecon Corp, Palo Alto, CA) were used and baited daily with 10  $\mu$ l of the neat compound(s) applied to a 5  $\times$  9 rubber septum (Thomas Scientific, Philadelphia, PA) in the bottom of the trap (1982 experiment) or baited every four days with 350 mg of a 20% (W/W) formulation of the compounds in plasticized polyvinyl chloride (PVC) (Tenneco, Piscataway,

NJ) (1983 experiment)<sup>8</sup>. One trap was used for each treatment. From September 10 to October 1, 1982, traps were hung from tree branches around the edge of an open field about 2 m from the ground and at least 10 m apart. From August 17 to October 3, 1983, traps were hung 10 cm from the ground on metal stakes 7 m apart. The stakes were in a row along a powerline cut through woods and traps were rotated 1 position each day. Extensive field-testing of the *P. maculiventris* pheromone was conducted simultaneously in other parts of the research center using sticky traps and traps made from transparent cylindrical plastic containers by cutting 9 cm diameter holes in opposite sides and covering each hole with an inwardly projecting screen funnel<sup>9</sup>. Trapped insects were removed daily from all the traps.

**Results.** When foraging yellowjackets came within about 50 cm downwind of a pheromone-baited trap, they often flew straight into the trap. While field-testing the artificial attractant pheromone of *P. maculiventris*, 334 *V. maculifrons* workers were caught in 7 pheromone-baited sticky traps and 15 *V. maculifrons* workers were caught in 7 control traps, from June 7 through October 3, 1983. In June, 1 *V. maculifrons* queen was caught in a pheromone-baited sticky trap and 3 *V. maculifrons* queens were captured alive in plastic traps baited with pheromone in PVC. Single workers of *Vespa crabro* and *Dolichovespula maculata* were caught in pheromone-baited traps, and single workers of *D. arenaria* and *V. crabro* were caught in the control traps.

In tests of single and paired compounds, traps hung from tree branches in 1982 that were baited with linalool or with linalool, (+)- $\alpha$ -terpineol, or (-)- $\alpha$ -terpineol mixed with (*E*)-2-hexenal caught significantly more *V. maculifrons* workers than traps baited with the other compounds (table). In 1983, using traps hung from metal stakes and repositioned daily, the linalool/(*E*)-2-hexenal and pheromone baited-traps caught significantly more *V. maculifrons* workers than all other compounds tested, including linalool. The trap baited with (*E*)-2-hexenyl crotonate, an ester similar to those attractive to western yellowjackets, caught only three *V. maculifrons* workers but did catch the only worker of *V. vidua* collected during the

study. Geraniol (an isomer of linalool) by itself or combined with (*E*)-2-hexenal was unattractive to yellowjackets. Although an apiary was located about 100 m away from the 1983 test site, only three honeybees were trapped; two in the linalool-baited trap and one in the nerol-baited trap. Spined soldier bugs were caught only in traps baited with the five component artificial pheromone; none were attracted to the single or paired compounds.

**Discussion.** Linalool and  $\alpha$ -terpineol act synergistically with (*E*)-2-hexenal to attract workers of the eastern yellowjacket, *V. maculifrons*. The number of *V. maculifrons* workers trapped was much lower than the numbers of *V. pensylvanica* workers trapped in the western United States with ester attractants; however, earlier tests with esters used at least 25 times more attractant than our tests<sup>2,3</sup>. Using ester attractants, Grothaus et al.<sup>4</sup> caught moderate numbers of *V. squamosa* followed in decreasing order by *V. vidua* and *V. maculifrons* at the Beltsville research center. We caught no *V. squamosa* in any of our traps at the research center. Four *V. maculifrons* queens were captured in pheromone-baited traps during June of 1983, suggesting that mixtures of (*E*)-2-hexenal with linalool or  $\alpha$ -terpineol will also attract foraging queens of this species.

Despite the abundance and importance of *V. maculifrons* in the eastern United States, the foraging behavior of this species is not well known<sup>10</sup>, although chemotaxis is apparently partly responsible for the success of foraging workers<sup>11</sup>. Foraging *V. maculifrons* workers are more solitary than *V. germanica* workers<sup>12</sup> and prefer living prey<sup>10</sup>. The eastern yellowjacket is reportedly a major predator of the fall webworm, *Hyphantria cunea*<sup>13</sup>. Rau<sup>14</sup> observed *V. maculifrons* queens entering the burrows of various species of ground-nesting bees which is interesting because linalool is a component of the aggregation pheromone in some of these bees<sup>15,16</sup>. There are no records of spined soldier bugs being preyed upon by *V. maculifrons* and we never saw this or any yellowjacket attack *P. maculiventris* around pheromone traps.

The eastern yellowjacket apparently does not use the pheromone of male spined soldier bugs as a kairomone specifically to find the bugs or their prey because (*E*)-2-hexenal/linalool is at least as attractive as the pheromone. (*E*)-2-Hexenal, known as leaf aldehyde, has been found in at least 30 plant families<sup>17</sup>. Damaged leaves enzymatically convert linolenic acid to leaf aldehyde and release much more of this compound than intact plants<sup>17,18</sup>. Linalool is also ubiquitous in the plant kingdom and is a basic precursor of monoterpenes in plants<sup>19</sup>. Glucosides of linalool and other monoterpene alcohols occur in many plants and when leaves are macerated the nonvolatile glucosides are hydrolyzed releasing the volatile alcohols<sup>17,20</sup>. Therefore, we hypothesize that the combination of  $\alpha$ -terpineol or linalool and (*E*)-2-hexenal constitutes a damaged-leaf-odor that *V. maculifrons* uses as a general kairomone to find insects feeding on leaves.

Attractiveness of volatile compounds to *Vespa maculifrons* workers

Compound(s)	Number <i>V. maculifrons</i>	
	1982	1983
<i>P. maculiventris</i> pheromone	—	26 a
Linalool*	9 a	2 b
Terpinen-4-ol*	0 b	1 b
(+)- $\alpha$ -Terpineol*	1 b	3 b
( <i>E</i> )-2-Hexenal*	1 b	3 b
Benzyl alcohol*	—	0 b
(-)- $\alpha$ -Terpineol	1 b	—
Geraniol	0 b	0 b
Nerol	—	3 b
( <i>E</i> )-2-Hexenal	—	2 b
( <i>Z</i> )-3-Hexenal	—	2 b
Terpinen-4-ol/( <i>E</i> )-2-hexenal	1 b	—
(+)- $\alpha$ -Terpineol/( <i>E</i> )-2-hexenal	8 a	—
(-)- $\alpha$ -Terpineol/( <i>E</i> )-2-hexenal	7 a	—
Linalool/( <i>E</i> )-2-hexenal	10 a	32 a
Linalool/( <i>E</i> )-2-hexenol	—	2 b
Linalool/( <i>Z</i> )-3-hexenol	—	3 b
Geraniol/( <i>E</i> )-2-hexenal	—	1 b
( <i>E</i> )-2-Hexenyl crotonate	—	3 b
Blank control	—	0 b

Numbers in a column followed by different letters are significantly different ( $p < 0.05$ , chi-square). An asterisk indicates a *P. maculiventris* aggregation pheromone component. A dash in a column under a year indicates that the treatment was not tested in that year. Single females of two parasitoids of *P. maculiventris*, *Euclytia flava* and *Hemidyda aurata* (Diptera: Tachinidae), were caught in the trap baited with (+)- $\alpha$ -terpineol/(*E*)-2-hexenal.

- 1 We thank A. S. Menke of the Systematic Entomology Laboratory, USDA, for identifying the yellowjackets. Mention of a company name does not imply endorsement by the US Department of Agriculture.
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## Platelet insulin receptor determination in non-insulin dependent diabetes mellitus

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**Summary.** The platelet membrane insulin receptors of healthy and non-insulin dependent (type 2) diabetic patients were studied. Receptor number and affinity proved to be decreased in type 2 diabetes mellitus. The changes in platelet insulin receptor characteristics are in good correlation with the alterations reported in other tissues or cells. The possible role of these phenomena in the pathogenesis of disturbed platelet function in diabetics needs further investigation.

**Key words.** Diabetes mellitus, type 2; platelets, human diabetic; insulin receptor, platelet; platelet insulin receptor.

The demonstration and characterization of human platelet membrane insulin receptors were done by Hajek et al.<sup>1</sup> in 1979. In this paper 125-I-insulin binding of isolated platelets from healthy persons and from non-insulin dependent diabetics (type 2 diabetes mellitus) was compared.

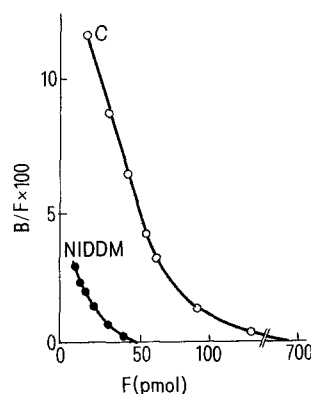
**Materials and methods.** The healthy control group was composed of 19 persons (male: 10, female: 9); the mean age was 46.4 years. 13 diabetic patients were studied (male: 8, female: 5). They all had the non-insulin dependent form (type 2) of the disease, without severe obesity; none of them received insulin or oral antidiabetic drugs. In the test period the patients had good metabolic control. Their mean age was 53.4 years. The same platelet isolation procedure was performed in the control as in the diabetic group, although the platelet population in diabetes mellitus might be more heterogeneous.

All of the samples were collected during fasting, between 08.00 and 09.00 h. Our method was based upon the one that Hajek<sup>1</sup> had used. Briefly: Blood was collected by vein puncture, and ACD (citric acid, dextrose) solution was used as anticoagulant. Platelet-rich plasma was obtained by centrifugation (15 min, 300 × g, 4°C). After washing in Tyrode (0, 35% BSA) solution, platelets were resuspended in 'HEPES-buffer' (0.1 M HEPES, 0.12 mM NaCl, 1.2 mM MgSO<sub>4</sub>, 2.5 mM KCL, 10 mM glucose, 1 mM EDTA, 1% BSA, pH 8.00). For the insulin binding assays a commercially available monoiodinated 125-I-insulin was used (MTA 1-RBO-22). 125-I-insulin was added to the suspension of platelets (insulin 100 pg/tube, 1–2 × 10<sup>8</sup> platelets/tube) in 'HEPES' buffer in the presence or absence of unlabeled insulin (0–10 µg/ml). The mixtures were incubated at room temperature for 150 min. After the incubation the platelets were separated by centrifugation, and radioactivity of the platelet sediments was determined.

The insulin binding in the presence of 10 µg/ml unlabeled insulin was regarded as nonspecific (2, 1–3, 0%). The results were evaluated by the graphical method of Scatchard<sup>2</sup>, using the same conventions (i.e. the negative cooperativity model) as Hajek et al.<sup>1</sup> had done. According to their method only one association constant was given derived from the dissociation constant (CD<sub>50</sub>) of the whole curve. For a better comparison the half-maximal displacement values were also determined.

**Results.** The Scatchard-plots derived from our data are in the figure.

The curvilinear shape of the plots appears to be consistent with either the presence of two classes of platelet insulin receptors with different numbers and affinity, or a single population according to the negative cooperativity model. The results shown here, of course, are not suitable for making a decision owing to the difficulties of explanation and interpretation of Scatchard-plots. (In order to make an adequate comparison of our results with those of Hajek et al.<sup>1</sup> we had to use the same mathematical conventions as they had done.) However, the remarkable decrease in the number of affinity of platelet insulin binding sites in the diabetic patients could be clearly demonstrated. In the table the results are shown in numerical form.



Platelet insulin receptor, Scatchard plots. 'F' is the quantity of free, 'B' the quantity of bound insulin. C: control group. NIDDM: non-insulin dependent diabetes mellitus.

Platelet insulin receptor study

	Receptor/ cell ± SD	1/CD <sub>50</sub> ± 10 <sup>7</sup> nmol/l	Half-maximal displacement nmol/l ± SD
Control	420 ± 116 (110 ± 23)	2.72 ± 0.75	5.98 ± 2.48
Diabetes mellitus type 2 (13 patients)	30 ± 11	1.20 ± 0.42	3.11 ± 1.73